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(71) Applicant *(for all designated States except US)*: PROTEUS MOLECULAR DESIGN LIMITED [GB/GB]; Proteus House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)* : FISHLEIGH, Robert, Vincent [GB/GB]; Bradley Smithy Cottage, Gurnett, Macclesfield, Cheshire SK11 0HD (GB). ROBSON, Barry [GB/GB]; The Old Bakery, 22A Town Street, Marple Bridge, Marple, Cheshire SK6 5AA (GB). GREANEY, Paul, John [GB/GB]; Flat No. 6, 5 Lancaster Road, Didsbury, Manchester M20 8TY (GB).

(74) Agents: HILDYARD, Edward, Martin et al.; Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).

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(54) Title: POLYPEPTIDE ANTIGENS OF MYCOBACTERIUM BOVIS

(57) Abstract

The invention provides a synthetic polypeptide having at least one antigenic property of the MPB-70 protein of at least one strain of *Mycobacterium bovis*, said polypeptide of Formula (I) comprising an amino acid sequence selected from Formulae Seq. ID Nos 1-14: Seq. ID No 1: X-Glu-Tyr-Ala-Ala-Ala-Asn-Pro-Thr-Gly-Pro-Ala-Ser-Val-Gln-Gly-Y; Seq. ID No 2: X-Met-Ser-Gln-Asp-Pro-Val-Ala-Val-Ala-Ala-Ser-Asn-Asn-Pro-Y; Seq. ID No 3: X-Pro-Glu-Leu-Thr-Thr-Leu-Thr-Ala-Ala-Leu-Ser-Gly-Gln-Leu-Asn-Pro-Gln-Y; Seq. ID No 4: X-Val-Val-Ala-Gly-Gln-Thr-Ser-Pro-Ala-Asn-Val-Y; Seq. ID No 5: X-Pro-Gln-Val-Asn-Leu-Val-Asp-Thr-Leu-Asn-Ser-Gly-Gln-Tyr-Ser-Val-Y; Seq. ID No 6: X-Phe-Ala-Pro-Thr-Asn-Ala-Ala-Phe-Ser-Y; Seq. ID No 7: X-Pro-Ala-Ser-Thr-Leu-Asp-Glu-Leu-Thr-Asn-Y; Seq. ID No 8: X-Gly-Thr-Arg-Thr-Gln-Leu-Gln-Gly-Ala-Ser-Val-Thr-Y; Seq. ID No 9: X-Gly-Thr-Arg-Gln-Thr-Leu-Gln-Gly-Ala-Ser-Val-Thr-Y; Seq. ID No 10: X-Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-Thr-Gln-Leu-Gln-Gly-Y; Seq. ID No 11: X-Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-Gln-Thr-Leu-Gln-Gly-Y; Seq. ID No 12: X-Thr-Gly-Gln-Gly-Asn-Ser-Leu-Lys-Val-Gly-Asn-Ala-Asp-Y; Seq. ID No 13: X-Ala-Thr-Val-Tyr-Met-Ile-Asp-Ser-Val-Leu-Met-Pro-Pro-Ala-Y; and Seq. ID No 14: X-Pro-Gln-Val-Asn-Leu-Val-Asp-Thr-Leu-Asn-His-Gly-Gln-Tyr-Ser-Y; wherein X and Y may each independently be absent or independently be one or more amino acid residues, with the proviso that when present they do not provide or form part of an epitope of the MPB-70 protein of at least one strain of *Mycobacterium bovis* which is contiguous with the sequence to which X and Y are attached, X or Y optionally being terminated by a functionalised coupling moiety. The synthetic polypeptides according to the invention may be used to elicit the production of antibodies to the MPB-70 protein.

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POLYPEPTIDE ANTIGENS OF MYCOBACTERIUM BOVIS

The present invention relates to synthetic polypeptides. It particularly relates to synthetic polypeptides which emulate the antigenic properties of specific regions of a protein of Mycobacterium bovis, the causative agent of bovine tuberculosis (BTB).

BTB is a major disease of cattle worldwide and costs the cattle industry many tens of millions of dollars each year. In addition to the health effects on cattle, M. bovis is closely related to M. tuberculosis which affects man, and there are health risks to humans from infected cattle. Campaigns to eradicate BTB have drastically reduced the incidence of the disease but the lack of sensitivity and specificity of current BTB tests have hampered total eradication.

Diagnosis of M.bovis in live cattle currently relies on identifying potentially infected animals by screening skin-test reactions to subcutaneous injections of bovine PPD (Purified Protein Derivative of a mycobacterial culture filtrate) tuberculin. The PPD produces a swelling at the site of injection which is monitored on a subjective basis by the examining veterinarian over a period of three days. If TB is suspected, every animal in the herd is tested and positive reactors are either segregated or slaughtered. The test must then be repeated at three- to six-monthly intervals to detect any fresh reactors.

Although this has been the method of choice for 100 years it suffers from several disadvantages and has continued to be used only because of the lack of a better test. The drawbacks include:

a) A lack of sensitivity depending on environmental conditions; figures quoted range from 65-82%;

b) A lack of specificity due to cross-reactions occurring with other environmental mycobacteria; the antigenic components of PPD responsible for the observed reaction remain largely uncharacterized;

c) The test is unable to distinguish animals with the active disease from animals sensitised to M.bovis;.

d) The test results in sensitisation of the animal with the result that repeat tuberculin testing cannot be carried out for at least 60 days;

e) There is a three day wait for the result; and

f) The test is expensive to carry out since it requires two visits by the veterinarian.

A satisfactory serological test for diagnosis of bovine TB has so far eluded researchers. Although many different types have been studied, none has proved as sensitive and specific as the tuberculin test. More recently, in vitro cellular assays and M.bovis-specific DNA probes have been developed which exhibit more acceptable sensitivities and specificities. However, these suffer from other disadvantages such as cost or need for sophisticated equipment which precludes their use in field surveys and less developed countries.

The major requirement for diagnosis of bovine tuberculosis is a highly specific, sensitive and non-subjective test. A simple serological test is the preferred option since this would be suitable for field surveys, abattoir monitoring and use in developing countries. In addition such a test would:

a) Give a more rapid result;

b) Cut down on cost;

c) Allow for better standardisation of test procedures;

d) Speed up eradication campaigns by detecting larger numbers of infected cattle; and

e) Not compromise the immune status of the animal.

The test should also differentiate between active disease and an anamnestic response to tuberculin.

To date, no effective vaccine against BTB has been developed and the tuberculin test remains the only effective method for controlling the disease. BCG (Bacille Calmette-Guerin), an avirulent strain of M. bovis that is used to vaccinate humans against TB, has been tested as a possible vaccine in cattle against BTB [Berggen, S.A. (1981), Br. Vet. J. 137, 88-94]. In several large-scale studies, BCG was found to offer no effective protection against BTB. There is a major requirement therefore for an effective vaccine against BTB.

Recent work has suggested that MPB-70, a major protein antigen of M. bovis and M. bovis/BCG strains, may distinguish M. bovis from other mycobacteria commonly found in the environment, (see for example WO 89/09261, CSIRO). It is known that there is amino acid sequence variation between some strains of M. bovis but the protein is readily distinguished from other antigens of M. bovis.

An object of the present invention is the development of synthetic polypeptides which can elicit the production of antibodies to MPB-70.

Our invention provides a synthetic polypeptide having at least one antigenic property of the MPB-70 protein of at least one strain of Mycobacterium bovis, said polypeptide of Formula I comprising an amino acid sequence selected from Formulae Seq. ID Nos: 1-14:-

Seq. ID No: 1

X-Glu-Tyr-Ala-Ala-Ala-Asn-Pro-Thr-Gly-Pro-Ala-Ser
-Val-Gln-Gly-Y;

Seq. ID No: 2

X-Met-Ser-Gln-Asp-Pro-Val-Ala-Val-Ala-Ala-Ser-Asn-
Asn-Pro-Y;

Seq. ID No: 3

X-Pro-Glu-Leu-Thr-Thr-Leu-Thr-Ala-Ala-Leu-Ser-Gly-Gln
Leu-Asn-Pro-Gln-Y;

Seq. ID No: 4

X-Val-Val-Ala-Gly-Gln-Thr-Ser-Pro-Ala-Asn-Val-Y;

Seq. ID No: 5

X-Pro-Gln-Val-Asn-Leu-Val-Asp-Thr-Leu-Asn-Ser-
Gly-Gln-Tyr-Ser-Val-Y;

Seq. ID No: 6

X-Phe-Ala-Pro-Thr-Asn-Ala-Ala-Phe-Ser-Y;

Seq. ID No: 7

X-Pro-Ala-Ser-Thr-Leu-Asp-Glu-Leu-Thr-Asn-Y;

Seq. ID No: 8

X-Gly-Thr-Arg-Thr-Gln-Leu-Gln-Gly-Ala-
Ser-Val-Thr-Y;

Seq. ID No: 9

X-Gly-Thr-Arg-Gln-Thr-Leu-Gln-Gly-Ala-
Ser-Val-Thr-Y;

Seq. ID No: 10

X-Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-Thr-Gln-
Leu-Gln-Gly-Y;

Seq. ID No: 11

X-Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-Gln-Thr-
Leu-Gln-Gly-Y;

Seq. ID No: 12

X-Thr-Gly-Gln-Gly-Asn-Ser-Leu-Lys-Val-Gly-
Asn-Ala-Asp-Y;

Seq. ID No: 13

X-Ala-Thr-Val-Tyr-Met-Ile-Asp-Ser-Val-
Leu-Met-Pro-Pro-Ala-Y; and

Seq.ID No.14

X-Pro-Gln-Val-Asn-Leu-Val-Asp-Thr-Leu-Asn-His-

wherein X and Y may each independently be absent or independently be one or more amino acid residues, with the proviso that when present they do not provide or form part of an epitope of the MPB-70 protein of at least one strain of Mycobacterium bovis which is contiguous with the sequence to which X and Y are attached, X or Y optionally being terminated by a functionalised coupling moiety, as defined hereinafter.

Peptides according to Formulae Seq. ID Nos: 1-14 above without X and Y being present are useful, for example, in the production of antibodies to MPB-70. Such peptides are especially effective when conjugated to a carrier molecule. However, when X or Y are present they may be any length but preferably less than 20 amino acids, more preferably less than 10, e.g. 1 to 6. It will of course be appreciated that the sequences according to Formulae Seq. ID Nos: 1-14 may constitute a protein with X and Y being major portions of the protein with the antigenic sequence being, for example, part of an exposed loop on a globular protein.

It is preferred that if X and Y are present they are relatively short sequences, typically 1 to 3 residues long. In most instances, either Y is absent and X is 1 or 2 residues long, or X is absent and Y is 1 or 2 residues long e.g. -Cys or -Gly-Cys. Such short N-terminal or C-terminal extensions provide alternative sites for coupling to a carrier. Preferably if X or Y is 1 residue, that residue provides an alternative site for coupling to a carrier. Preferably if X or Y is 2 residues, one residue provides an alternative site for coupling to a carrier and the other residue is any amino acid acting as a spacer, preferably Gly.

Advantageously, X is absent and Y is -Gly-Cys in Seq. ID Nos: 2-12 and 14, and X is Ala and Y is preferably -Cys in Seq. ID No: 1. However, it is preferable that X is Cys-Gly- and Y is absent in Seq. ID No: 13 since in this case N-terminal conjugation is better suited to the sequence. When glycine residues are present in X or Y they act as spacers. It is envisaged that heterobifunctional coupling moieties may also function as suitable spacers. The residue within X or Y that provides an alternative site for coupling to a carrier may be a natural amino acid which has a suitable functional group for coupling to a carrier, e.g. Cys, Lys, Tyr. Alternatively, the residue may be an amino acid that has been chemically modified (i.e. "functionalised") so that a functional group is introduced specifically for the purpose of facilitating coupling to a carrier. Examples of functional groups useful in coupling to a carrier include thiol, amino, hydrazino or hydrazide, and aldehyde or masked aldehyde groups.

Preferred polypeptide sequences according to the invention were chosen on the basis of their topographical similarity to one or more antigenic determinants of the MPB-70 protein of M. bovis.

Peptides according to the invention may be synthesised by any suitable method, for example by use of either standard 9-fluorenyl-methoxycarbonyl (F-Moc) chemistry (see, for example, Atherton, E. and Sheppard, R. C. (1985) J. Chem. Soc. Chem. Comm. 165) or standard t-butyloxycarbonate (t-Boc) chemistry. The correctness of the structure and the level of purity, which will normally be in excess of 85%, should be carefully checked. Various chromatographic analyses, including high performance liquid chromatography, and spectrographic analyses, may for example be employed for this purpose.

In order to facilitate high yields and good purity, peptides made using Fmoc chemistry may be N-terminally acetylated and/or C-terminally amidated, and such

modifications are included within the scope of the present invention (Stuber, W., Knolle, J. & Breipohl, G. (1989). Synthesis of peptide amides by Fmoc-solid-phase peptide synthesis on acid labile anchor groups. Int. J. Pept. & Prot. Res., 34, 215-221).

All the sequences herein are stated using the standard I.U.P.A.C. three-letter-code abbreviations for amino acid residues defined as follows: Gly-Glycine, Ala-Alanine, Val-Valine, Leu-Leucine, Ile-Isoleucine, Ser-Serine, Thr-Threonine, Asp-Aspartic acid, Glu-Glutamic acid, Asn-Asparagine, Gln-Glutamine, Lys-Lysine, His-Histidine, Arg-Arginine, Phe-Phenylalanine, Tyr-Tyrosine, Trp-Tryptophan, Cys-Cysteine, Met-Methionine and Pro-Proline.

Polypeptides according to the invention may be used to raise antibodies which will cross-react with MPB-70 proteins produced by a wide range of M. bovis strains. Our analyses have shown that since the conformational/topographic/electrostatic properties of polypeptides according to the invention are such that they are highly likely to elicit the production of antibodies which will cross-react with MPB-70 proteins from several or many strains, further advantages may arise from combining several variant polypeptides in a larger polypeptide. Such a polypeptide may have the general Formula (II):

$$[L_a\text{-}F]_m\text{-}[L_b\text{-}G]_n\text{-}L_c \qquad (II)$$

wherein F and G may each independently be a polypeptide according to any one of Formulae Seq. ID Nos: 1-14, L is a linking sequence, a, b and c are each independently 0 or 1 and m and n are each positive numbers e.g. between 1 and 10 inclusive. L is preferably a short, conformationally flexible section of polypeptide chain such as, for example and without limit Gly-Gly-Gly-Gly-Gly (Seq. ID No: 15), Gly-Pro-Gly-Pro-Gly-Pro (Seq. ID No: 16) or Gly-Ser-Ala-Gly-Ser-Gly-Ala (Seq. ID No: 17). It should be clear that each repeat may optionally have

a different variant of a polypeptide according to the invention.

Polyvalent determinant analogues as defined by Formula II are referred to as pseudohomopolyvalent, wherein variants of essentially the same determinant analogue are repeated in a single polypeptide chain. In addition, simple homopolyvalent polypeptide immunogens, which contain multiple copies of the same variant of one of the determinant analogues according to any one of Formulae I to II, are also effective, and are also included within the scope of the present invention.

Pseudohomopolyvalent immunogenic polypeptides are particularly valuable as vaccines, where they elicit the production of a range of (neutralising) antibodies with a similar but non-identical underlying specificity, which between them cross-react with MPB-70 protein from a wider range of M. bovis strains, and are thus more effective at conferring protective immunity. There are also advantages in constructing heteropolyvalent polypeptides which contain one or more copies, in any order, of one of the polypeptides according to the present invention and one or more other polypeptide analogues or determinant analogues. Such polypeptides, which are provided for in the present invention, have the general Formula (III):

$$L_d\text{-}[G\text{-}L]_m\text{-}F\text{-}[L\text{-}G]_n\text{-}L_e \qquad \text{(III)}$$

wherein F is a polypeptide according to any one of Formulae Seq. ID Nos: 1-14, G is a polypeptide according to any one of Formulae Seq. ID Nos: 1-14 or other sequence, m and n are each positive numbers e.g. between 1 and 10 inclusive, and d and e are each independently 0 or 1. "L" is preferably a short, conformationally flexible section of polypeptide chain such as, for example and without limit Gly-Gly-Gly-Gly-Gly (Seq. ID No: 15), Gly-Pro-Gly-Pro-Gly-Pro (Seq. ID No: 16) or Gly-Ser-Ala-Gly-Ser-Gly-Ala (Seq. ID No: 17).

It is to be understood that any antigenic

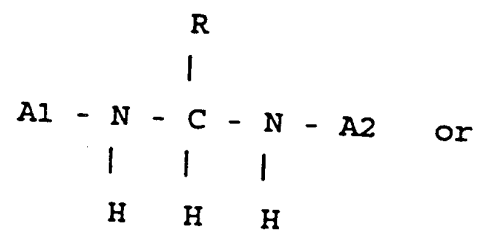
significant subfragments and/or antigenically significant variants of the above-identified polypeptide sequences which retain the general form and function of the parent polypeptide are included within the scope of this invention. For example the N-terminal Pro-Glu of Seq. ID No: 3 may be omitted in some instances yet the peptide will still retain its general form and function. The substitution of any of the specific residues by residues having comparable conformational and/or physical properties, including substitution by rare amino acids (e.g. D-stereoisomers) or synthetic amino acid analogues, is included within the scope of the invention. For example, substitution of a residue by another in the same Set, as defined below, is included within the ambit of the invention; Set 1 - Ala, Val, Leu, Ile, Phe, Tyr, Trp and Met; Set 2 - Ser, Thr, Asn and Gln; Set 3 - Asp and Glu; Set 4 - Lys, His and Arg; Set 5 - Asn and Asp; Set 6 - Glu and Gln; Set 7 - Gly, Ala, Pro, Ser and Thr. D-stereoisomers of all amino acid types, may be substituted, for example, D-Phe, D-Tyr and D-Trp.

Embodiments of the invention, in which X and/or Y are present may independently include one or more segments of protein sequence with the ability to act as a T-cell epitope. For example, segments of amino acid sequence of the general formula 1-2-3-4, where 1 is Gly or a charged amino acid (e.g. Lys, His, Arg, Asp or Glu), 2 is a hydrophobic amino acid (e.g. Ile, Leu, Val, Met, Tyr, Phe, Trp, Ala), 3 is either a hydrophobic amino acid (as defined above) or an uncharged polar amino acid (e.g. Asn, Ser, Thr, Pro, Gln, Gly), and 4 is a polar amino acid (e.g. Lys, Arg, His, Glu, Asp, Asn, Gln, Ser, Thr, Pro), appear to act as T-cell epitopes in at least some instances (Rothbard, J.B. & Taylor, W.R. (1988); "A sequence pattern in common to T-cell epitopes." The EMBO Journal 7(1): 93-100).

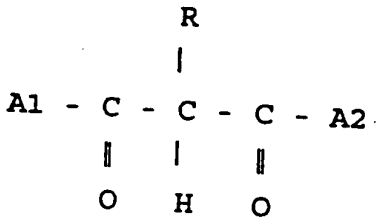
Similarly segments can be of the sequence 1'-2'-3'-4'-5', wherein 1' is equivalent to 1 as defined earlier, 2' to 2, 3' and 4' to 3, and 5' to 4 (ibid). Both forms

are included within the scope of the present invention and one or more T-cell epitopes (preferably less than five) may be incorporated into a polypeptide according to any one of Formulae I to III. The or each epitope may be of the type defined above or may be of other structure and may be separated by spacer segments of any length or composition (preferably less than five amino acid residues in length) and comprise for example residues selected from Gly, Ala, Pro, Asn, Thr, Ser or polyfunctional linkers such as non-α amino acids. It is possible for a C- or N-terminal linker to represent a complete protein, thus obviating the possible need for conjugation to a carrier protein.

Also included within the scope of this invention are analogues of the polypeptide according to Formulae Seq. ID Nos: 1-14 in which X or Y are or include a "retro-inverso" amino acid, i.e. a bifunctional amine or bifunctional carboxyl. For example an analogue according to the invention and containing a retro-inverso amino acid may have the formula:



or



where R is any amino acid side chain group, e.g. a glycine side chain, and A1 and A2 are preferably each at least one synthetic polypeptide according to the invention or other peptide sequence, e.g. having desirable antigenic properties (but not necessarily the same) attached by its N- or C-terminal end. T-cell epitopes may

optionally be included in A1 and/or A2 as discussed earlier.

Retro-inverso modification of peptides involves the reversal of one or more peptide bonds to create analogues more resistant than the original molecule to enzymatic degradation and offer one convenient route to the generation of branched immunogens which contain a high concentration of epitope for a medium to large immunogen. The use of these compounds in large-scale solution synthesis of retro-inverso analogues of short-chain biologically active peptide is of particular interest.

It should be noted that analogues incorporating retro-inverso amino acid derivatives cannot be made directly using a recombinant DNA system. However, the basic analogues can be synthesised by recombinant means and they can then be purified and chemically linked to the retro-inverso amino-acids using standard techniques of peptide/organic chemistry. A practical and convenient novel procedure for the solid-phase synthesis on polyamide-type resin of retro-inverso peptides has been described recently [Gazerro, H., Pinori, M. & Verdini, A.S. (1990); "A new general procedure for the solid-phase synthesis of retro-inverso peptides"; a section of "Innovation and Perspectives in Solid Phase Synthesis" Ed. Roger Epton, SPCC (UK) Ltd, Birmingham, UK].

The polypeptides are optionally linked to a carrier molecule, either through chemical groups within the polypeptides themselves or through the additional amino acids comprising the X and Y groups (if present). The carrier molecule may be separated from the polypeptides by one or more additional amino acids acting as spacers, in order to render the polypeptides optimal for their immunological function. Many linkages are suitable and include for example use of the side chains of Tyr, Cys and Lys residues. Suitable carriers include, for

example, tetanus toxoid, cholera toxin and its B subunit, ovalbumin, soybean trypsin inhibitor, muramyl dipeptide and analogues thereof, and Braun's lipoprotein although other suitable carriers will be readily apparent to the skilled person. For example, multiple antigen peptides may be used such as those comprising a polylysyl core, e.g. heptalysyl, bearing reactive amino termini. Polypeptide antigens according to the invention may be reacted with, or synthesised on, the amino termini and different polypeptide antigens may be reacted with the same core or carrier.

The mode of coupling the polypeptide to the carrier will depend on the nature of the materials to be coupled. For example, a lysine residue in the carrier may be coupled to a C-terminal or other cysteine residue in a polypeptide by treatment with N-γ- maleimido-butyryloxy-succinimide (Kitagawa, T. & Ackawa, T. (1976) J. Biochem. 79, 233). Other coupling reactions and reagents have been described in the literature.

The polypeptides, either alone or linked to a carrier molecule, may be administered by any route (e.g. parenteral, nasal, oral, rectal, intra-vaginal), with or without the use of conventional adjuvants (such as aluminium hydroxide or, in the case of laboratory animals, Freund's complete or incomplete adjuvants) and/or other immunopotentiating agents. In our co-pending PCT application No. PCT/GB93/00716 filed 6 April 1993 (claiming a priority date of 7 April 1992), we disclose non-ionic surfactant vesicles which may be used advantageously as adjuvants with peptides according to the present invention. The invention also includes formulation of polypeptides according to the invention in slow-release forms, such as a sub-dermal implant or depot comprising, for example, liposomes (Allison, A.C. & Gregoriadis, G. (1974) Nature (London) 252, 252) or biodegradable microcapsules manufactured from co-polymers of lactic acid and glycolic acids (Gresser, J.

D. and Sanderson, J. E. (1984) in "Biopolymer Controlled Release Systems" pp 127-138, Ed. D. L. Wise).

In some circumstances it will be advantageous to immunise with a cocktail containing (i) a given polypeptide conjugated to more than one type of carrier molecule, and/or (ii) more than one kind of polypeptide conjugated to the same carrier molecule. Moreover, any of the polypeptides, their conjugates, and cocktails thereof may be administered in any suitable adjuvant or delivery system, and more than one adjuvant or delivery system may be combined to form a so-called "super-cocktail". Preferred adjuvants and delivery systems include aluminium hydroxide, liposomes, micelles, niosomes, ISCOMS and whole-cell or components of microbial animal vaccines.

It is to be understood that the polypeptides according to the invention may be synthesised by any conventional method, either directly using manual or automated peptide synthesis techniques as mentioned above, or indirectly by RNA or DNA synthesis and conventional techniques of molecular biology and genetic engineering. Such techniques may be used to produce hybrid proteins containing one or more of the polypeptides inserted into another polypeptide sequence.

Another aspect of the present invention therefore provides a DNA molecule coding for at least one synthetic polypeptide according to the invention, preferably incorporated into a suitable expression vector replicable in microorganisms or in mammalian, insect, plant, fungal or other cells. The DNA may also be part of the DNA sequence for a longer product e.g. the polypeptides may be expressed as parts of other proteins into which they have been inserted by genetic engineering. One practical guide to such techniques is "Molecular cloning: a laboratory manual" by Sambrook, J., Fritsch, E.F. and Maniatis, T. (2nd Edition, 1989).

Polypeptides according to the invention may be used either alone or linked to an appropriate carrier, as:

(a) Peptide vaccines, for use to prevent infection by one or more strains of M. bovis;

(b) As ligands in assays of, for example, serum from M. bovis positive subjects;

(c) As antigens for in vitro cellular bioassays e.g. the gamma-interferon assay which detects gamma-interferon released in response to specific antigen in a whole blood culture;

(d) As quality control agents in testing, for example, binding levels of antibodies raised against the polypeptides; and

(e) As immunogenic agents for the generation of monoclonal or polyclonal antibodies by immunisation of an appropriate animal, such antibodies being of use for (i) the scientific study of the M. bovis, (ii) as diagnostic agents, e.g. as part of histochemical reagents, and (iii) for the passive immunisation of subjects. The invention further provides for genetically engineered forms or sub-components, especially V_H regions, of antibodies raised against the polypeptides, and of bovinised forms of antibodies initially raised against the polypeptides in other animals, using techniques described in the literature.

In respect of detection and diagnosis, of M. bovis or antibodies against M. bovis, the skilled person will be aware of a variety of immunoassay techniques known in the art, inter alia, sandwich assay, competitive and non-competitive assays and the use of direct and indirect labelling.

A further aspect of the invention provides a method of detecting M. Bovis or antibodies against M. Bovis or or antigen binding fragments thereof, which comprises incubating a sample of tissue or body fluid of a mammal with at least one polypeptide according to the invention and determining whether, and if desired the extent to which and/or rate at which cross reaction between said sample and said polypeptide occurs.

A further aspect of the invention provides a kit for detecting M. bovis or antibodies against M. bovis which comprises at least one synthetic polypeptide according to the invention. In some instances it will be desirable to include a mixture of polypeptides according to the invention in the kit.

Such kits may also comprise support means (e.g. plastic, e.g. polystyrene, latex or red blood cells) and/or means of detecting binding of antibodies or antigen binding fragments to the synthetic polypeptide (e.g. fluorescent, radio or enzymatically labelled anti-Ig antibodies).

The preparation of polyclonal or monoclonal antibodies, recombinant forms particularly adapted to the species of interest, e.g. bovinised forms of antibodies (see, for example, Thompson K. M. et al (1986) Immunology 58, 157-160), single domain antibodies (see, for example, Ward, E. S., Gussow, D., Griffiths, A. D., Jones, P. and Winter, G. (1989) Nature 341, 544-546), which bind specifically to a synthetic polypeptide according to the present invention, may be carried out by conventional means and such antibodies are considered to form part of this invention. Antibodies according to the invention are, inter alia, of use in a method of diagnosing mammalian M. bovis infection which comprises incubating a sample of tissue or body fluid of a mammal with an effective amount of antibody or antigen binding fragment thereof as described herein and determining whether, and if desired the extent to which and/or rate at which, cross-reaction between said sample and said antibody occurs. Diagnostic kits which contain at least one of said antibodies also form part of this invention. Such kits may comprise one or more of the following: support means; antibodies or antigen binding fragments thereof according to the invention; at least one synthetic polypeptide according to the invention; and means for detecting binding of antibodies or antigen binding fragments to said synthetic polypeptide.

The use of synthetic polypeptides or antibodies or antigen binding fragments thereof according to the invention the detection of M. Bovis or antibodies against M. Bovis on histological sections also form part of this invention.

Antibodies raised by immunisation using a synthetic polypeptide according to the invention can be used to raise anti-idiotypic antibodies which also form part of this invention.

A further aspect of the invention provides synthetic p -ypeptides and antibodies or antigen binding fragments thereof as defined above for use in stimulating the mammalian immune system for the therapy or prophylaxis of mammalian M. bovis infection; and for the preparation of medicaments suitable for such uses. Also included are pharmaceutical, especially vaccine, compositions containing, as active ingredient, at least one polypeptide or polypeptide-carrier conjugate as described herein in association with one or more pharmaceutically acceptable adjuvants, and/or excipients. Additionally are included pharmaceutical, especially vaccine, compositions containing, as active ingredient, an antibody or antigen binding fragment thereof which binds specifically to a synthetic polypeptide or polypeptide coupled to a carrier according to the present invention, in association with one or more pharmaceutically acceptable adjuvants, and/or excipients. The compositions may be formulated for oral, rectal, nasal or especially parenteral administration.

The invention further provides a method of stimulating the mammalian immune system for the therapy or prophylaxis of mammalian M. bovis infection which comprises administering an effective amount of a polypeptide or an antibody or antigen binding fragment thereof as hereinbefore defined to a mammalian subject (e.g. to deer, cattle, pigs, sheep, badgers, goats and possums; in particular to deer and cattle) either in

isolation or in combination with other agents for the treatment of tuberculosis.

The following are non-limiting Examples are intended to illustrate the invention.

EXAMPLE 1

Synthesis of Peptides

The following peptides were synthesised using standard Fmoc solid phase chemistry (Atherton, E & Sheppard, R C, 1985; J. Chem. Soc. Commun. 165-166).

1a Glu-Tyr-Ala-Ala-Ala-Asn-Pro-Thr-Gly-Pro-Ala-Ser-Val-Gln-Gly-Cys
(Seq. ID No: 1 in which X is absent and Y is Cys);

2a Met-Ser-Gln-Asp-Pro-Val-Ala-Val-Ala-Ala-Ser-Asn-Asn-Pro-Gly-Cys
(Seq. ID No: 2 in which X is absent and Y is Gly-Cys);

3a Pro-Glu-Leu-Thr-Thr-Leu-Thr-Ala-Ala-Leu-Ser-Gly-Gln-Leu-Asn-Pro-Gln-Gly-Cys
(Seq. ID No: 3 in which X is absent and Y is Gly-Cys);

4a Val-Val-Ala-Gly-Gln-Thr-Ser-Pro-Ala-Asn-Val-Gly-Cys
(Seq. ID No: 4 in which X is absent and Y is Gly-Cys);

6a Phe-Ala-Pro-Thr-Asn-Ala-Ala-Phe-Ser-NH_2
(Seq. ID No: 6 in which X and Y are absent);

7a Pro-Ala-Ser-Thr-Leu-Asp-Glu-Leu-Thr-Asn-NH_2
(Seq. ID No: 7 in which X and Y are absent);

8a Gly-Thr-Arg-Thr-Gln-Leu-Gln-Gly-Ala-Ser-Val-Thr-NH_2
(Seq. ID No: 8 in which X and Y are absent);

9a Gly-Thr-Arg-Gln-Thr-Leu-Gln-Gly-Ala-Ser-Val-Thr-NH_2
(Seq. ID No: 9 in which X and Y are absent);

10a Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-Thr-Gln-Leu-Gln-Gly-NH_2
(Seq. ID No: 10 in which X and Y are absent);

11a Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-Gln-Thr-Leu-Gln-Gly-NH_2
(Seq. ID No: 11 in which X and Y are absent);

12a Thr-Gly-Gln-Gly-Asn-Ser-Leu-Lys-Val-Gly-Asn-Ala-Asp-NH_2
(Seq. ID No: 12 in which X and Y are absent);

13a Ala-Thr-Val-Tyr-Met-Ile-Asp-Ser-Val-Leu-Met-Pro-Pro-Ala-NH_2
(Seq. ID No: 13 in which X and Y are absent);

and

14a Pro-Gln-Val-Asn-Leu-Val-Asp-Thr-Leu-Asn-His-Gly-Gln-Tyr-Ser-OH
(Seq. ID No: 14 in which X and Y are absent);

EXAMPLE 2

Diagnosis of bovine tuberculosis by serological assay

The peptide antigens from Example 1 were tested against sera from known M. bovis-positive and M. bovis-negative cattle using an enzyme immunoassay (ELISA). Polystyrene microtitre plates (Nunc MaxiSorp) were coated with antigen diluted in carbonate buffer (pH 9.6) overnight at 4°C. (The concentration of antigen solution was previously determined by ELISA checkboard assay). The antigen solution was discarded and the plates were blocked with 5% milk powder diluted in phosphate buffered saline (pH 7.3) with 0.05% Tween 20 (PBST) for one hour at room temperature. The block solution was discarded and the test sera (dilution 1 in 200 using 10% normal rabbit serum in PBST) were added to

the plate prior to incubation for one hour at room temperature. The test sera were discarded and the plates were washed five times with phosphate buffered saline (pH 7.3; PBS). Rabbit anti-bovine IgG conjugated to peroxidase (diluted 1 in 1000 using 10% normal rabbit serum in PBST) was added to the plates and they were incubated for one hour at room temperature. The conjugate was then discarded and the plates were washed five times with PBS prior to addition of the substrate, 3,3', 5,5'-tetramethylbenzidine (TMB). The reaction was allowed to proceed for 30 minutes in the dark and then stopped with sulphuric acid. The plates were then read on an ELISA plate reader at a wavelength of 450 nm.

The peptides were tested singly and in combination against 60 bovine sera from tuberculin skin test-positive or bacteriologically confirmed M.bovis-positive animals (confirmed at post-mortem) and 60 sera from skin test-negative animals. Results were compared with those for avian PPD, bovine PPD and whole MPB70 antigen.

Trial 1

Peptides 1a, 2a, 3a, 4a, 6a, 8a, 11a, 12a, combination of peptides 1a+2a, 3a+4a, 6a+8a, 11a+12a and MPB70 and ovarian and bovine PPD were run against serum samples from 60 skin test positive cattle and 60 skin test negative cattle using the ELISA protocol described above (TABLE 1). The 60 skin test negative cattle came from herds clear of bovine tuberculosis for at least five years.

Trial 2

Peptides 3a, 4a, 14a, 6a, 7a, 8a, 9a, 10a and 13a and bovine PPD were run against 60 serum samples from cattle with confirmed bovine tuberculosis (from post-mortem) and 60 skin test negative cattle (TABLE 2).

For each antigen the sample was run in duplicate and the mean O.D. value calculated. Sensitivity and specificity values for each antigen were then calculated. The sensitivity is a measure of the antigen's ability to predict skin test positive animals (Trial 1) or M.bovis-positive animals (Trial 2) and the specificity is a measure of the antigen's ability to predict skin test negative animals. The cut-off value between a positive and a negative result was calculated as the mean of the negative group plus two standard deviations.

Results

All peptides shared high specificities. Peptides BTB-6 and BTB-8 showed the highest sensitivities against the skin test-positive sera and also against M.bovis-positive sera; against M.bovis -positive sera, both showed greater sensitivity than PPD.

Table 1: Results obtained using tuberculin skin test positive and negative sera (60 of each)

Antigen	Sensitivity (% of sera tested)	Specificity (% of sera tested)
1a	19	98.3
2a	26.7	100
1a + 2a	26.7	100
3a	81.7	98.3
4a	85	100
3a + 4a	45	98.3
6a	83.3	98.3
8a	93	98.3
6a + 8a	83.3	96.7
11a	60	98.3
12a	55	100
11a + 12a	63.3	100
MPB 70	49	100
Avian PPD	90	98.3
Bovine PPD	93.3	95

Table 2: Results obtained using sera from animals confirmed as M.bovis positive or M.bovis negative (60 of each)

Antigen	Sensitivity (% of sera tested)	Specificity (% of sera tested)
3a	40	98.3
4a	55	100
6a	100	98.3
7a	28.3	98.3
8a	96.7	98.3
9a	18.3	98.3
10a	30	98.3
13a	31.7	98.3
14a	25	100
Bovine PPD	88.3	96.7

EXAMPLE 3

Diagnosis of bovine tuberculosis by cellular assay

A peptide having the sequence: Val-Val-Ala-Gly-Gln-Thr-Ser-Pro-Ala-Asn-Val-Gly-Cys (Seq. ID No: 4 in which X is absent and Y is -Gly-Cys) was synthesised as described in Example 1.

The peptide antigen is tested against heparinized venous blood samples from known M. bovis-positive and M. bovis-negative cattle using a whole-blood culture and gamma-interferon enzyme immunoassay system. The antigen and 1.0 ml aliquots of blood are dispensed into a 24-well tissue culture tray and incubated at 37°C in a humidified atmosphere of 5% carbon dioxide in air. After 24 hours the plasma is removed and assayed for gamma-interferon by a sandwich enzyme immunoassay. The plasma is incubated in microtitre trays coated with anti-bovine gamma-interferon monoclonal antibody. Gamma-interferon which binds in the wells is then detected with different anti-bovine gamma interferon monoclonal antibodies conjugated to peroxidase. The plates are washed and incubated with the substrate, 3,3', 5,5'-tetramethylbenzidine (TMB), for 30 minutes. The reaction is stopped with sulphuric acid and the plates are read on an ELISA plate reader at a wavelength of 450nm.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PROTEUS MOLECULAR DESIGN LIMITED
(B) STREET: PROTEUS HOUSE, LYME GREEN BUSINESS PARK
(C) CITY: MACCLESFIELD
(D) STATE: CHESHIRE
(E) COUNTRY: ENGLAND
(F) POSTAL CODE (ZIP): SK11 0JL

(A) NAME: FISHLEIGH, ROBERT VINCENT
(B) STREET: BRADLEY SMITHY COTTAGE, GURNETT
(C) CITY: MACCLESFIELD
(D) STATE: CHESHIRE
(E) COUNTRY: ENGLAND
(F) POSTAL CODE (ZIP): SK11 0HD

(A) NAME: ROBSON, BARRY
(B) STREET: THE OLD BAKERY, 22A TOWN STREET, MARPLE BRIDGE
(C) CITY: MARPLE
(D) STATE: CHESHIRE
(E) COUNTRY: ENGLAND
(F) POSTAL CODE (ZIP): SK6 5AA

(A) NAME: GREANEY, PAUL JOHN
(B) STREET: FLAT 6, 5 LANCASTER ROAD
(C) CITY: DIDSBURY
(D) STATE: MANCHESTER
(E) COUNTRY: ENGLAND
(F) POSTAL CODE (ZIP): M20 8TY

(ii) TITLE OF INVENTION: SYNTHETIC POLYPEPTIDES

(iii) NUMBER OF SEQUENCES: 17

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9215514.2
(B) FILING DATE: 22-JUL-1992

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9311273.8
(B) FILING DATE: 01-JUN-1993

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Glu Tyr Ala Ala Ala Asn Pro Thr Gly Pro Ala Ser Val Gln Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Gln Asp Pro Val Ala Val Ala Ala Ser Asn Asn Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Pro Glu Leu Thr Thr Leu Thr Ala Ala Leu Ser Gly Gln Leu Asn Pro
1 5 10 15

Gln

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Val Val Ala Gly Gln Thr Ser Pro Ala Asn Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Pro Gln Val Asn Leu Val Asp Thr Leu Asn Ser Gly Gln Tyr Ser Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Phe Ala Pro Thr Asn Ala Ala Phe Ser
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Pro Ala Ser Thr Leu Asp Glu Leu Thr Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gly Thr Arg Thr Gln Leu Gln Gly Ala Ser Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Gly Thr Arg Gln Thr Leu Gln Gly Ala Ser Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Pro Ala Asn Val Val Gly Thr Arg Thr Gln Leu Gln Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Pro Ala Asn Val Val Gly Thr Arg Gln Thr Leu Gln Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Thr Gly Gln Gly Asn Ser Leu Lys Val Gly Asn Ala Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ala Thr Val Tyr Met Ile Asp Ser Val Leu Met Pro Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Pro Gln Val Asn Leu Val Asp Thr Leu Asn His Gly Gln Tyr Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Gly Gly Gly Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Gly Pro Gly Pro Gly Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Gly Ser Ala Gly Ser Gly Ala
1 5

Claims

1. A synthetic polypeptide having at least one antigenic property of the MPB-70 protein of at least one strain of Mycobacterium bovis, said polypeptide of Formula I comprising an amino acid sequence selected from Formulae Seq. ID Nos: 1-14:-

X-Glu-Tyr-Ala-Ala-Ala-Asn-Pro-Thr-Gly-Pro-Ala-Ser-Val-Gln-Gly-Y (Seq. ID No: 1);

X-Met-Ser-Gln-Asp-Pro-Val-Ala-Val-Ala-Ala-Ser-Asn-Asn-Pro-Y (Seq. ID No: 2);

X-Pro-Glu-Leu-Thr-Thr-Leu-Thr-Ala-Ala-Leu-Ser-Gly-Gln Leu-Asn-Pro-Gln-Y (Seq. ID No: 3);

X-Val-Val-Ala-Gly-Gln-Thr-Ser-Pro-Ala-Asn-Val-Y (Seq. ID No: 4);

X-Pro-Gln-Val-Asn-Leu-Val-Asp-Thr-Leu-Asn-M-Gly-Gln-Tyr-Ser-N-Y

in which and M is Ser and N is Val (Seq. ID No: 5); or M is His and N is absent (Seq. ID No: 14);

X-Phe-Ala-Pro-Thr-Asn-Ala-Ala-Phe-Ser-Y (Seq. ID No: 6);

X-Pro-Ala-Ser-Thr-Leu-Asp-Glu-Leu-Thr-Asn-Y (Seq. ID No: 7);

X-Gly-Thr-Arg-P-Q-Leu-Gln-Gly-Ala-Ser-Val-Thr-Y

in which P is Thr and Q is Gln (Seq. ID No: 8); or P is Gln and Q is Thr (Seq. ID No: 9);

X-Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-S-T-Leu-Gln-Gly-Y;

in which S is Thr and T is Gln (Seq. ID No: 10); or S is Gln and T is Thr (Seq. ID No: 11);

Seq. ID No: 12

X-Thr-Gly-Gln-Gly-Asn-Ser-Leu-Lys-Val-Gly-Asn-Ala-Asp-Y;

Seq. ID No: 13

X-Ala-Thr-Val-Tyr-Met-Ile-Asp-Ser-Val-Leu-Met-Pro-Pro-Ala-Y; and

wherein X and Y may each independently be absent or independently be one or more amino acid residues, with the proviso that when present they do not provide or form part of an epitope of the MPB-70 protein of at least one strain of Mycobacterium bovis which is contiguous with the sequence to which X and Y are attached; X or Y optionally being terminated by a functionalised coupling moiety.

2. A synthetic polypeptide as claimed in claim 1 in which any X and Y groups present each comprise 1-6 amino acids.

3. A synthetic polypeptide as claimed in claim 1 or claim 2 in which either Y is absent and X is 1-2 amino acids or X is absent and Y is 1-2 amino acids.

4. A synthetic polypeptide as claimed in any one of the previous claims in which any X and Y groups present each are 1-2 amino acids, one of these amino-acids providing a site suitable for attachment to a carrier, the other, if present, being Gly.

5. A synthetic polypeptide as claimed in any one of claims 1-4 in which an amino-acid within any X and Y groups present has been modified to provide a functional

group for coupling to a carrier.

6. A synthetic polypeptide as claimed in claim 1 of general Formula (II):

$$[L_a\text{-}F]_m\text{-}[L_b\text{-}G]_n\text{-}L_c \qquad (II)$$

wherein F and G may each independently be a polypeptide according to any one of the sequences of Formula I, L is a linking sequence, a, b and c are each independently 0 or 1 and m and n are each positive numbers.

7. A synthetic polypeptide as claimed in claim 1 of general Formula (III):

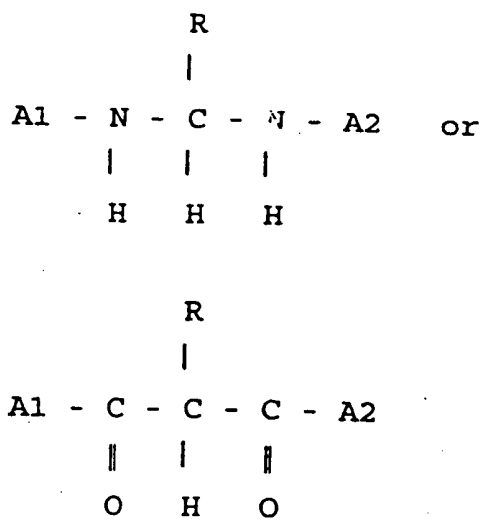
$$L_d\text{-}[G\text{-}L]_m\text{-}F\text{-}[L\text{-}G]_n\text{-}L_e \qquad (III)$$

wherein F is a polypeptide according to any one of the sequences of Formula I, G is a polypeptide according to any one of the sequences of Formula I or other sequence, L is a linking sequence, m and n are each positive numbers and d and e are each independently 0 or 1.

8. A synthetic polypeptide which comprises an antigenically significant subfragment or variant of a polypeptide as claimed in any one of claims 1 to 5.

9. A synthetic polypeptide as claimed in any one of the preceding claims additionally comprising at least one T-cell epitope.

10. An analogue of a synthetic polypeptide as claimed in any one of the preceding claims in which X or Y are or include a retro-inverso amino acid said analogue having the formula



where R is any amino acid side chain group, and A1 and A2, which may be the same or different, are both amino acid sequences attached by their N- or C-terminal ends, at least one of A1 and A2 being at least one synthetic polypeptide as claimed in any one of the preceding claims.

11. A synthetic polypeptide as claimed in any one of claims 1 to 10 coupled to a vaccine carrier.

12. A vaccine comprising at least one synthetic polypeptide as claimed in any one of claims 1 to 11 effective to promote immune protection in a mammal against at least one strain of Mycobacterium bovis, and a physiologically acceptable adjuvant and/or excipient.

13. An antibody or antigen binding fragment thereof which specifically binds to a synthetic polypeptide as claimed in any one of claims 1 to 11.

14. Anti-idiotypic antibodies which are specific to an antibody or antigen binding fragment thereof as claimed in claim 13.

15. A DNA molecule coding for at least one synthetic polypeptide as claimed in any one of claims 1 to 10.

16. A kit for detecting Mycobacterium bovis or antibodies against Mycobacterium bovis which comprises at least one synthetic polypeptide as claimed in any one of claims 1 to 11, and optionally comprising support means and/or means for detecting binding of antibody or antigen binding fragments to said polypeptide.

17. A diagnostic kit for detecting Mycobacterium bovis or antibodies against Mycobacterium bovis which comprises at least one antibody or antigen binding fragment thereof as claimed in claim 13, and optionally comprising one or more of: support means; at least one synthetic polypeptide as claimed in any one of claims 1-11; and means for detecting binding of antibody or antigen binding fragment to said polypeptide.

18. Use of a synthetic peptide as claimed in any one of claims 1-11 for stimulating the mammalian immune system for the therapeutic or prophylactic treatment of mammalian Mycobacterium bovis infection.

19. A pharmaceutical composition containing, as active ingredient, at least one polypeptide as claimed in any one of claims 1 to 11 in association with one or more pharmaceutically acceptable adjuvants and/or excipients.

20. A pharmaceutical composition containing, as active ingredient an antibody or antigen binding fragment thereof as claimed in claim 13 in association with one or more pharmaceutically acceptable adjuvants and/or excipients.

21. A method of stimulating the mammalian immune system for the therapy or prophylaxis of mammalian Mycobacterium bovis infection which comprises administering an effective amount of a polypeptide as claimed in any one of claims 1 to 11 to a mammalian subject, either in isolation or in combination with other agents for the treatment of

tuberculosis.

22. A method of detecting Mycobacterium bovis or antibodies against Mycobacterium bovis or antigen binding fragments thereof, which comprises incubating a sample of tissue or body fluid of a mammal with at least one polypeptide as claimed in any one claims 1 to 11 and determining whether, and if desired the extent to which and/or rate at which, cross reaction between said sample and said polypeptide occurs.

23. A method of diagnosing mammalian Mycobacterium bovis infection which comprises incubating a sample of tissue or body fluid of a mammal with an effective amount of an antibody or antigen binding fragment thereof as claimed in claim 13 and determining whether, and if desired the extent to which and/or rate at which, cross-reaction between said sample and said antibody or antigen binding fragment thereof occurs.

24. A process for the manufacture of a synthetic polypeptide having at least one antigenic property of the MPB70 protein of at least one strain of Mycobacterium bovis, said polypeptide of Formula I comprising an amino acid sequence selected from Formulae Seq. ID Nos: 1-14:-

X-Glu-Tyr-Ala-Ala-Ala-Asn-Pro-Thr-Gly-Pro-Ala-Ser
-Val-Gln-Gly-Y (Seq. ID No: 1);

X-Met-Ser-Gln-Asp-Pro-Val-Ala-Val-Ala-Ala-Ser-Asn-
Asn-Pro-Y (Seq. ID No: 2);

X-Pro-Glu-Leu-Thr-Thr-Leu-Thr-Ala-Ala-Leu-Ser-Gly-Gln
Leu-Asn-Pro-Gln-Y (Seq. ID No: 3);

X-Val-Val-Ala-Gly-Gln-Thr-Ser-Pro-Ala-Asn-Val-Y
(Seq. ID No: 4);

X-Pro-Gln-Val-Asn-Leu-Val-Asp-Thr-Leu-Asn-M-Gly-Gln-Tyr-Ser-N-Y

in which and M is Ser N is Val (Seq. ID No: 5); or M is His and N is absent (Seq. ID No: 14);

X-Phe-Ala-Pro-Thr-Asn-Ala-Ala-Phe-Ser-Y (Seq. ID No: 6);

X-Pro-Ala-Ser-Thr-Leu-Asp-Glu-Leu-Thr-Asn-Y (Seq. ID No: 7);

X-Gly-Thr-Arg-P-Q-Leu-Gln-Gly-Ala-Ser-Val-Thr-Y

in which P is Thr and Q is Gln (Seq. ID No: 8); or P is Gln and Q is Thr (Seq. ID No: 9);

X-Pro-Ala-Asn-Val-Val-Gly-Thr-Arg-S-T-Leu-Gln-Gly-Y;

in which S is Thr and T is Gln (Seq. ID No: 10); or S is Gln and T is Thr (Seq. ID No: 11);

Seq. ID No: 12

X-Thr-Gly-Gln-Gly-Asn-Ser-Leu-Lys-Val-Gly-Asn-Ala-Asp-Y;

Seq. ID No: 13

X-Ala-Thr-Val-Tyr-Met-Ile-Asp-Ser-Val-Leu-Met-Pro-Pro-Ala-Y; and

wherein X and Y may each independently be absent or independently be one or more amino acid residues, with the proviso that when present they do not provide or form part of an epitope of the MPB-70 protein of at least one strain of Mycobacterium bovis which is contiguous with the sequence to which X and Y are attached; X or Y optionally being terminated by a functionalised coupling moiety,

the process comprising the steps of coupling the residues using chemical, biological and/or recombinant techniques known per se and isolating the polypeptide.

25. A process for the manufacture of an antibody or antigen binding fragment thereof as claimed in claim 13, which process comprises immunising a mammal with a synthetic polypeptide as claimed in any one of claims 1 to 11 and isolating the antibody formed or cells which produce the antibody.

26. Use of an antibody or antigen binding fragment as claimed in claim 13 for stimulating the mammalian immune system for the therapeutic or prophylactic treatment of mammalian Mycobacterium bovis infection.

27. A method of stimulating the mammalian immune system for the therapy or prophylaxis of mammalian Mycobacterium bovis infection which comprises administering an effective amount of an antibody or antigen binding fragment as claimed in claim 13 to a mammalian subject, either in isolation, or in combination with other agents for the treatment of tuberculosis.

28. Use of a synthetic polypeptide as claimed in any one of claims 1 to 12 in the preparation of a medicament for stimulating the mammalian immune system for the therapeutic or prophylactic treatment of mammalian Mycobacterium bovis infection.

29. Use of an antibody or antigen binding fragment as claimed in claim 13 in the preparation of a medicament for stimulating the mammalian immune system for the therapeutic or prophylactic treatment of mammalian Mycobacterium bovis infection.